With Col. D. Bruce's Compliments,
Royal Army Medical College,
Millbank, S.W.

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BY COLONEL SIR DAVID BRUCE, C.B., F.R.S., A.M.S.; AND CAPTAINS A. E. HAMERTON, D.S.O., AND H. R. BATEMAN, R.A.M.C.

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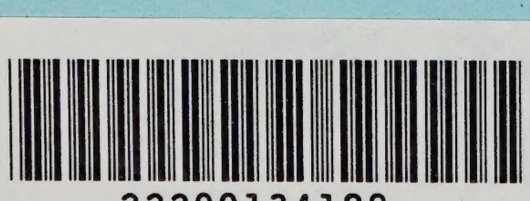
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Experiments to Ascertain if the Domestic Fowl of Uganda may Act as a Reservoir of the Virus of Sleeping Sickness (Trypanosoma gambiense).

By Colonel Sir DAVID BRUCE, C.B., F.R.S., A.M.S.; and Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, R.A.M.C. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10).

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Introduction.

Birds of various species are very numerous on the shores of Lake Victoria. Of these, cormorants, darters, herons (African grey and purple, and other species), ibises (glossy and sacred), fish-eagles, weaver birds (various species), terns (various species), gulls, geese (Egyptian and spur-winged), plovers (various species), pratincoles, storks, kingfishers and gallinules are the most common. These birds all inhabit areas where the *Glossina palpalis* are numerous, and some evidence is forthcoming that in Nature the fly feeds on avian blood.*

In view of the continued infectivity of the fly in the depopulated areas of the Lake-shore, it is clear that every effort should be made to ascertain the source of such infectivity. Search for a reservoir of the virus of Sleeping Sickness at once suggests itself, and to those acquainted with the fauna of the Lake-shore, an avian host would be included amongst the various species requiring investigation.

Domestic fowls were employed in these experiments. They are readily obtained, easily handled, and the flies feed greedily on them. One species of trypanosome may in Nature only affect one species of avian host; therefore, it follows that whatever the result of this series of experiments may be, that result is only applicable to the fowl. The Commission trust that no wider significance will be attached to the conclusions set forth at the end of this paper: in other words, because a Uganda fowl is or is not a reservoir of *Trypanosoma gambiense*, it does not follow that a cormorant, or other bird, is or is not a reservoir. It would have been better for this reason to have experimented with one or other of the various species of wild Lake-shore birds. The difficulties were, however, too great at the time these experiments were carried out. It is hoped that this paper will stimulate rather than discourage other workers to carry on this line of investigation.

* 'Roy. Soc. Proc.,' B, 1910, vol. 82, p. 496, Table IV.

In order to discover whether Uganda fowls can act as a reservoir of the virus of Sleeping Sickness, the Commission asked themselves the following questions :—

1. Can fowls be infected with the virus of Sleeping Sickness by the bites of laboratory-bred and laboratory-infected *Glossina palpalis ?*

2. Can fowls transmit the virus of Sleeping Sickness (*Trypanosoma gambiense*) to clean laboratory-bred *Glossina palpalis ?* If this is possible, can the flies so infected convey the virus to normal susceptible animals ?

An answer in the affirmative to those two questions would throw suspicion on a possible avian host. To decide these points, the following experiments were devised and carried out :—

Can Fowls be Infected with the Virus of Sleeping Sickness (Trypanosoma gambiense) by the Bites of Laboratory-bred and Laboratory-infected Glossina palpalis ?

The method adopted to infect the fowls was carried out as follows :—Clean *Glossina palpalis*, hatched out in the laboratory, were given their first feeds on a monkey whose blood contained many *Trypanosoma gambiense.* An interval of at least 24 hours was then allowed to elapse before the infected flies were first fed on the fowl. The fowl was then fed on, for varying periods, by these flies. To make certain the flies were infective, their infectivity was usually tested by their being allowed to bite normal susceptible animals during the experiment. Finally, to discover if the fowl had become infected with *Trypanosoma gambiense* by the bites of the *Glossina palpalis* :—

(*a*) The fowl's blood was examined frequently throughout the experiment for *Trypanosoma gambiense.*

(*b*) The fowl was killed and its centrifuged heart or jugular vein blood was carefully examined microscopically for *Trypanosoma gambiense.*

(*c*) Two or more cubic centimetres of the centrifuged blood were injected into a normal susceptible animal on one or more occasions during the experiment.

Two typical experiments are given in detail.

Experiment 1915 *a*.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1909.				
Nov. 22—25	1—3	Flies fed on *T. gambiense*-infected monkey.		250 laboratory-bred *G. palpalis* used.
„ 26—Dec. 31	4—39	Flies fed on Cock 1926.	—	*T. gambiense*-infected flies found on Dec. 13, 14, 16, 17, 20, and 27.
1910.				
Jan. 1—10	40—49	Flies fed on normal monkey, Experiment 1999, to test their infectivity.		100 flies of this experiment survived to 50th day and were used for Experiment 1915*b*.
„ 11	50	Monkey, Experiment 1999, examined; blood showed *T. gambiense*.	+	Allowing 7 days for the incubation of *T. gambiense* in this monkey, it is seen that the flies infected it on Jan. 4—that is, 4 days after feeding on fowl.

Remarks.—On December 17, 1909, 1 c.c., and on December 22, 3 c.c. of the blood of cock, Experiment 1926, were injected into a normal monkey, Experiment 1954. This monkey remained healthy. On January 10, 1910, the cock was killed, 10 c.c. of its heart's blood were centrifuged, and the "buffy layer" carefully examined for *Trypanosoma gambiense*, with negative results. The whole of the 10 c.c. of blood obtained were then injected into two monkeys: 5 c.c. into monkey, Experiment 1954, and 5 c.c. into monkey, Experiment 2054. The blood of these two animals was examined frequently for a month after these injections. No trypanosomes were ever found and the monkeys remained healthy.

Experiment 2081.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910.				
Jan. 9—14 ...	1—5	Flies fed on *T. gambiense*-infected monkey.		120 laboratory-bred *G. palpalis* used.
„ 15—Feb. 2	6—24	Flies fed on cock, Experiment 2074.	—	Feb. 15, monkey, Experiment 2135, showed *T. gambiense* in its blood.
Feb. 3—7	25—29	Flies fed on normal monkey, Experiment 2135, to see if flies are infective.	+	Feb. 22, monkey, Experiment 2178, showed *T. gambiense* in its blood.
„ 8—14 ...	30—36	Flies again fed on cock, Experiment 2074.	—	Fowl, Experiment 2074, has therefore been fed on by infective *G. palpalis*.
„ 15—19 ...	37—41	Flies fed on normal monkey, Experiment 2178, to see if flies still infective.	+	

Remarks.—Cock, Experiment 2074, was killed on March 16, 1910; 10 c.c. of its heart's blood were taken and centrifuged and carefully examined for *Trypanosoma gambiense*, with negative results. The 10 c.c. were then injected, subcutaneously, into normal monkey, Experiment 2301. This monkey was examined bi-weekly up to April 15, 1910. No *Trypanosoma gambiense* were ever seen, and the monkey remained healthy.

The 13 experiments carried out on these lines are now given in tabular form, in Table I below :—

Table I.

No. of experiment.	No. of flies used.	No. of fowl fed on.	No. of infected flies found during experiment.	Result.	Remarks.
1915 *a*	250	Expt. 1926	7	Negative	Proved infective flies fed on this fowl.
1915 *b*	100	„ 2059	1	„	Proved infective flies fed on this fowl.
1915 *c*	50	„ 2182	—	„	Doubtful whether infected flies fed on this fowl.
1918	100	„ 1927	—	„	Proved infective flies fed on this fowl.
2081	120	„ 2074	2	„	Proved infective flies fed on this fowl.
2082	100	„ 2087	12	„	Proved infective flies fed on this fowl.
2208	100	„ 2209	0	„	Proved infective flies fed on fowl, but no flies dissected.
2325	160	„ 2334	2	„	Proved infective flies fed on fowl.
2362	110	„ 2363	7	„	Proved infective flies fed on fowl.
2365	300	„ 2336	0	„	Flies not dissected, but were proved infective.
2366	150	„ 2364	0	„	Flies not dissected, but were proved infective.
2390	180	„ 2367	7	„	Proved infective flies fed on fowl.
2395	100	„ 2396	1	„	Proved infective flies fed on fowl.

Remarks.—In these experiments it is seen that 1820 laboratory-bred and laboratory-infected *Glossina palpalis* were fed on 13 fowls, with uniformly negative results. With one exception, Experiment 1915 *c*, the flies were proved to be infective by feeding them on animals susceptible to *Trypanosoma gambiense* infection. That the fowls had not become infected with *Trypanosoma gambiense* was proved by carrying out the three tests given above, under headings *a*, *b*, and *c*. In Experiment 2325, the liver, spleen and bone-marrow of Fowl 2334 were also examined for trypanosomes, and 10 c.c. of the pooled pulp injected into a normal monkey. This monkey remained healthy.

A further series of experiments bearing on this question was carried out. These experiments are not so complete. The procedure was as follows :—

A varying number of laboratory-bred *Glossina palpalis* were fed for several days on *Trypanosoma gambiense*-infected antelope. The infected flies were then fed for varying periods on fowls. The fowls were then killed and 2 c.c. of their blood were injected into a normal monkey. The monkey's blood was examined for *Trypanosoma gambiense* bi-weekly for a month after the injection of the fowl's blood. The experiments are given in tabular form.

Table II.

Date.	Days of "cycle" the flies fed on fowl.	Experiment No. of fowl.	No. of flies used.	Did flies subsequently prove to be infective?	Result of inoculation of fowl's blood into normal monkeys.
1910.					
May 9—21	7—19th	Cock 2455	50	No	Negative
„ 13—31	4—22nd	„ 2471	60	Yes (18 p. c.)	„
„ 16—June 1	7—23rd	„ 2479	95	„ (2·1 p. c.)	„
„ 16— „ 1	4—20th	„ 2480	55	„ (15 p. c.)	„
June 2—11.....	10—19th	„ 2518	100	No	„
„ 2—14.....	5—17th	„ 2519	100	„	„
„ 2—17.....	8—23rd	„ 2519	100	Yes (8·8 p. c.)	„
„ 2—11.....	11—20th	„ 2519	100	No	„

Remarks.—The chief criticism against these experiments is the fact that the flies fed on the fowls during the earlier days of the experiments, when the *Glossina palpalis* are usually not infective. However, as results have been recorded by the Commission where *Glossina palpalis* have infected susceptible animals as early as the 18th day of an experiment, it has been decided to publish these experiments.

Can Fowls Transmit the Virus of Sleeping Sickness to clean Laboratory-bred Glossina palpalis? If this is possible, can the Flies so Infected convey the Virus to Normal Susceptible Animals?

In view of the results obtained by the first series of experiments, it would seem somewhat unnecessary to follow this question further.

It has so far been proved that the blood of fowls which have been fed upon by infected *Glossina palpalis* is non-infective when injected into susceptible animals. It has now to be ascertained if the blood of such fowls is also incapable of infecting the fly. This would constitute additional evidence against the fact that fowls act as a reservoir of *Trypanosoma gambiense.*

Cages of laboratory-bred and laboratory-infected *Glossina palpalis,* which were known to be infective, were fed on fowls for a varying number of days. Next, clean laboratory-bred flies were fed on these fowls for several days. The flies were then fed on normal susceptible animals (monkeys), in the endeavour to infect such animals with *Trypanosoma gambiense,* and so prove that the blood of the fowl was infective. The full details of three experiments are given below:—

Experiment 2018.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910. Jan. 4—10.....	1—6	Flies fed on Fowl 1927.		100 laboratory-bred *G. palpalis* used. Fowl, Experiment 1927, has been fed on for 29 days by *T. gambiense*-infected *G. palpalis*.
,, 11	7	Flies starved.		
,, 12—Feb. 23	8—50	Flies fed on Monkey 2061.	–	87 *G. palpalis* dissected throughout this experiment between the 11th and 55th days after flies' first feed on Fowl 1927. All were negative for flagellates. Monkey 2061 examined, with negative result, till March 7.

Experiment 2019.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910. Jan. 4—10.....	1—6	Flies fed on Fowl 1926.		100 laboratory-bred *G. palpalis* used. Fowl, Experiment 1926, has been fed on for 29 days by *T. gambiense*-infected *G. palpalis*.
,, 11	7	Flies starved.		
,, 12—Feb. 23	8—50	Flies fed on Monkey 2062.	–	80 *G. palpalis* dissected throughout this experiment between the 11th and 55th days after flies' first feed on Fowl 1926. All were negative for flagellates. Monkey 2062 was examined bi-weekly for *T. gambiense* until March 22; no trypanosomes were seen. Monkey remained healthy.

Experiment 2276.

Date.	Day of experiment.	Procedure.	Result.	Remarks.
1910.				
Mar. 7—12.....	1—5	Flies fed on Fowl 2087.		200 laboratory-bred flies used. Fowl, Experiment 2087, has been fed on for 18 days by *T. gambiense*-infected *G. palpalis*.
Mar. 13	6	Flies starved.		
„ 14—Apr. 16	7—40	Flies fed on Monkey 2344.	—	116 *G. palpalis* dissected throughout this experiment, between the 23rd and 42nd days after flies' first feed on Fowl 2087. All were negative for flagellates. Monkey 2344 was examined bi-weekly for *T. gambiense* till May 10; no trypanosomes ever seen. Monkey died of broken neck later.

Remarks.—It will be seen that 400 laboratory-bred *Glossina palpalis* fed on the three fowls, Experiments 1927, 1926, and 2087, subsequently failed to infect monkeys with the virus of Sleeping Sickness, and that all the 283 *Glossina* dissected throughout these experiments were negative for flagellates. This absence of flagellates in the flies is interesting, because Fowl 1927 and Fowl 1926 were both found to be naturally infected with a large avian trypanosome. It would thus seem that this fowl trypanosome does not undergo development in *Glossina palpalis*.

Conclusion.

The Uganda fowl cannot act as a reservoir of the virus of Sleeping Sickness.

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